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MEMORANDUM FOR Environmental Acquisition Support Branch (RDAR-MEE-E/ Mr. Joseph Dowden), Demilitarization & Environmental Technology Division, Energetics, Warheads and Manufacturing Technology Directorate, U.S. Army Armament Research, Development, and Engineering Center, Picatinny Arsenal, NJ 07806-5000

SUBJECT: Preliminary Occupational Exposure Level (OEL) for 2,4-dinitroanisole (DNAN)

1. Reference Statement of Work Military Interdepartmental Purchase Request # 1CDAT4D140, Title: Environmental Health Assessments for Ordnance Environmental Program New Materials, Mark S. Johnson, 5 April 2011.

2. Purpose. To provide a preliminary occupational exposure level (OEL) for DNAN.

3. Background. As part of the work effort, "Environmental Health Assessments for Ordnance Environmental Program New Materials," U.S. Army Public Health Command (USAPHC) conducted acute, subacute and subchronic oral toxicity studies for DNAN. In conjunction with these efforts, USAPHC was asked to develop an OEL for DNAN. Following conventional procedures, USAPHC conducted a review of the available toxicology database, selected the critical study and effect, identified the point of departure, and applied appropriate uncertainty factors (UF) (see enclosed Information Paper).

4. Recommendations. The present evaluation provides a preliminary OEL for DNAN of 0.09 mg/m^3, with a Skin notation. The use of the preliminary value is recommended until further data become available. The OEL is expressed as an 8-hour time-weighted average (TWA), and assumes an 8-hour work day and a 40-hour work week. The Skin notation indicates that skin absorption may be an important route of exposure for DNAN and that measures to prevent significant cutaneous absorption should be taken.

5. The Army Institute of Public Health point of contact is Dr. Emily May Lent, Toxicology Portfolio, Toxicity Evaluation Program. She may be contacted at DSN 584-7749, commercial 410-436-7749 or via email at emily.m.lent@us.army.mil.

FOR THE DIRECTOR:

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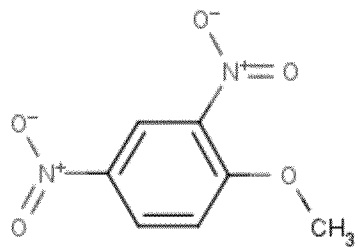
Information Paper: Preliminary Occupational Exposure level (OEL) for 2,4-Dinitroanisole

I. IDENTIFICATION

Chemical Name: 2,4-Dinitroanisole
Synonyms: 1-Methoxy-2,4-Dinitrobenzene; DNAN
CAS Number: 119-27-7
Molecular Formula: $C_7H_6N_2O_5$
Structural Formula:



II. CHEMICAL AND PHYSICAL PROPERTIES [1]

Physical State: Cream to yellow crystalline solid.
Odor Description: No data available
Molecular Weight: 198.133
Conversion Factors:
1 ppm = 8.10 mg/m^3
1 mg/m^3 = 0.12 ppm
Melting Point: 94.5°C (202.1°F)
Boiling Point: 206°C at 12 mm Hg
Vapor Pressure: No data available
Saturated Vapor Concentration: No data available
Flammability Limits: No data available
Flash Point: 181°C (357.8°F)
Autoignition Temperature: 347°C (656.6°F)
Specific Gravity: No data available
Vapor Density: No data available
Solubility: Slightly soluble in water; soluble in ethanol, ether, acetone, and benzene; very soluble in pyridine.
Stability: Stable
Reactivities and Incompatibilities: No data available

III. USES

DNAN was historically used as an explosive in warheads containing Amatol 40 and is currently being investigated as a replacement for 2,4,6-Trinitrotoluene (TNT) in melt-cast

ENCL

insensitive munitions (IM) formulations. DNAN is also used industrially in the synthesis of dyes and insect repellants.[1,2]

IV. TOXICOLOGY DATA

A. Acute Toxicity

1. *Oral Toxicity*

   $LD_{50\ (rat)}$ = 199 mg/kg[3]

2. *Eye Toxicity*

   Rabbit: mild irritant with reversibility in 48 hours[3]

3. *Skin Toxicity*

   a. Skin Irritation

   Rabbit: slight irritation with reversibility in 24-48 hours[3]

   b. Skin Sensitization

   Guinea pig: did not cause sensitization[3]

   c. Dermal Penetration

   Steady state flux of DNAN from powdered Composition B replacement (CBR-12, aka PAX-21) through dermatomed rat skin in static diffusion cells over six hours was determined to be 0.74 $\mu g/cm^2/hr$. For comparison, steady state flux of TNT from Composition B in the same system was 1.14 $\mu g/cm^2/hr$.[4]

4. *Inhalation*

   $LC_{50\ (rat,\ Sprague\text{-}Dawley,\ 4\ hr)} > 3\ g/m^3$. No mortalities were observed at the highest concentration achieved. DNAN was generated as an aerosol by dissolving in acetone and delivering through an air atomizing nozzle. Clinical signs of toxicity including decreased activity, breathing abnormalities and salivation were observed at this concentration.[5]

B. Genotoxicity/Mutagenicity

1. *In vitro*

   DNAN was evaluated in the Ames *Salmonella* test (TA98, TA100, TA102, TA1535, and TA1537), with and without metabolic activation (S9). DNAN was mutagenic in strain TA100 without activation.[6]

   Evaluation using Chinese Hamster Ovary (CHO) cells (AS52/XPRT) at concentrations of 0.0625 to 1.0 mg/ml with and without S9 activation indicated no mutagenic induction in the tested cells.[6]

2. *In vivo*

   DNAN was tested in the mouse micronucleus test. Male and female Swiss CD-1 mice were orally dosed with DNAN at 10 to 90 mg/kg. There was no significant increase in micronucleated cell frequency with DNAN treatment. The highest dose was considered to be toxic to the hematopoietic system, inducing a change in the ratio of polychromatic erythrocytes to normochromatic erythrocytes. DNAN was judged to have caused no chromosomal damage and to be non-mutagenic in the *in vivo* mouse bone marrow assay.[6]

C. Metabolism and Pharmacokinetics
No data available.

D. Developmental and Reproductive Toxicity
No data available.

E. Subacute Toxicity

1. *Oral Toxicity*

Male and female Sprague-Dawley rats (6/sex/group) were administered DNAN in corn oil at 0, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 mg/kg-day via oral gavage for 14 days. Male rats exposed to 100 mg/kg-day of DNAN exhibited reduced body mass and reduced testes mass. Increased kidney, liver, and spleen mass were observed in female rats given 50 and 100 mg/kg-day DNAN. In females, changes in hematology indicative of anemia, including decreased red blood cell count, hematocrit, and hemoglobin, and increased red cell distribution width were observed in the 100 mg/kg-day group. Increased alanine aminotransferase levels suggesting hepatocellular injury were also evident in female rats given 50 and 100 mg/kg-day DNAN.[7]

2. *Inhalation*

Male and female Sprague-Dawley rats (5/sex/group) were exposed to DNAN in acetone at nominal concentrations of 150, 500, and 1500 mg/m^3 of aerosol/vapor for 6 hours/day; 5 days/week, for a total of 11 exposures. All animals in the 1500 mg/m^3 and 8/10 animals in the 500 mg/m^3 group were found dead or euthanized during the exposure period. Clinical signs of toxicity observed prior to euthanasia included decreased food consumption, prostration, irregular gait, lethargy, head bobbing, poor condition, pale, backwards walking, labored breathing, and red nasal discharge. Animals exposed to 500 mg/m^3 gained less weight and consumed less feed during the first week of exposure than the acetone controls. Male rats exposed to 150 mg/m^3 had significantly decreased blood urea nitrogen (BUN) and increased kidney weight. Females in the 150 mg/m^3 had statistically significant decreases, relative to the acetone control group, in mean hemoglobin concentrations, mean corpuscular volume, and mean corpuscular hemoglobin and increases in mean absolute monocytes and liver weight. The urine of both male and female rats exposed to 150 mg/m^3 was darker than acetone controls. The only reported compound related microscopic finding was non-specific minimal metaplasia of laryngeal epithelium in rats exposed to 150 mg/m^3.[8]

F. Subchronic Toxicity

1. *Oral Toxicity*[7]

Male and female Sprague-Dawley rats were dosed with DNAN via oral gavage at 0, 1.25, 5, 20, and 80 mg/kg-day for 90 days. Mortality occurred in three male rats (days 50, 63, and 77) and one female rat (day 26) all from the 80 mg/kg-day dose group. Rats in the highest dose group (80 mg/kg-day) experienced lethargy, labored/rapid respiration, prostrate and/or recumbent posture, hunched posture, ear twitching, squinting, curled tail, and gait irregularities. A functional observation battery (FOB) and analysis of motor activity at week 13 indicated that rats given 80 mg/kg-day had altered neuromuscular function and decreased activity levels. In the 80 mg/kg-day group, female rats also had reduced sensorimotor responses while male rats had increased excitability responses.

Although food intake was similar among groups for male rats, animals from the 80 mg/kg-day dose group exhibited reduced body mass and a reduced food efficiency ratio. Female rats in the 80 mg/kg-day dose group also had a reduced food efficiency ratio, but had elevated food consumption at several time points during the study. Body mass did not differ among dose groups for female rats. Female rats in the 80 mg/kg-day dose group and male rats in the 20 mg/kg-day group produced higher volumes of urine with lower specific gravity. Despite the increase in volume, urine color was darker in the 20 and 80 mg/kg-day dose groups for both sexes.

Increased mean kidney, liver, and spleen mass were observed in male and female rats given 80 mg/kg-day DNAN. In male rats, increased mean kidney and liver mass were also noted in the 20 mg/kg-day dose group; however, the changes were not associated with treatment related microscopic abnormalities or alterations in clinical chemistry parameters. Decreased mass of the testes and epididymides as well as degeneration and atrophy of the testicular seminiferous tubules and aspermia were also observed in rats from the 80 mg/kg-day group. In females, changes in hematology indicative of anemia, including decreased red blood cell count, hematocrit, and hemoglobin, and increased red cell distribution width were observed in the 80 mg/kg-day group. A dose related increase in extramedullary hematopoeisis was noted in spleens of female rats at 20 and 80 mg/kg-day. Glial lesions within the cerebellum were noted in four rats (1 female/3 males) in the 80 mg/kg-day group.

2. *Inhalation*
   No data available.

G. Chronic Toxicity and Carcinogenicity
   No data available.

V. HUMAN USE AND EXPERIENCE

DNAN was used as a component of MYL louse powder (0.2% pyrethrins, 2.0% IN-930, 0.25% Phenol-S, 2.0% DNAN, and pyrophyllite inert diluent) until it was replaced by the longer acting DDT. In its use for control of human lice, MYL powder was applied to clothing at 30 g/suit, resulting in a dermal application of DNAN of 600 mg/man or 8.6 mg/kg. MYL powder was demonstrated to be safe through testing and use.[2] DNAN is currently being investigated as a replacement for TNT in a variety of insensitive munitions formulations. Limited air sampling in buildings where melt-pour and drilling operations for munitions containing DNAN are conducted revealed mean air levels at all operations of 1.19 mg/m^3 with a range of 0.03 to 8.5 mg/m^3.[9-10]

VI. RATIONALE

The available acute data for DNAN suggest that it is moderately toxic via the oral route and slightly toxic via inhalation. Occupational exposure to DNAN is likely to occur primarily through inhalation (aerosol, vapor) and potentially through dermal exposure. However, no long-term toxicity data are available for the inhalation route and no systemic data are available for the dermal route. Subacute inhalation data are available; however, a dose response assessment is not reliable since few individuals at more than one exposure group survived. Although this study indicated possible portal of entry effects, systemic effects similar to those observed following oral exposures were also observed, indicating that route-to-route extrapolation may be appropriate.[11] No chronic studies are available; one subchronic oral toxicity study was conducted in the rat. Extramedullary hematopoeisis (EMH) can be associated with anemia in female rats and as such was identified as the critical endpoint in this study.[12-13] Benchmark Dose Software (BMDS v.2.1.2) was used to fit mathematical models to the EMH incidence dose response data and calculate a lower-bound confidence limit on a dose corresponding to a 10% response rate ($BMDL_{10}$).[14-15] Three models were selected based on goodness-of-fit and statistical parameters and a mean $BMDL_{10}$ value of 2.3 mg/kg-day was calculated for use as the point of departure (POD). A total uncertainty factor (UF) of 300 was applied to the POD, which includes:[11,16-17]

Uncertainty Factor	*Value*	*Rationale*
Intraspecies (UF_H)	3	Default value of 10 was reduced to 3 based on the worker population being comprised of healthy adults.
Interspecies (UF_A)	10	Default value was used because only animal data in a single species are available. Additionally, PBPK data are lacking which would assist in developing a chemical specific adjustment factor (CSAF).
Subchronic to chronic (UF_S)	3	Default value of 10 was reduced to 3 based on comparison of dose response relationships between subacute and subchronic oral studies. The $BMDL_{10}$ for several anemia related parameters were approximately equal (subacute:subchronic = 0.88) across study durations. These data suggest that an increase in duration of exposure may not reduce the dose at which anemia related effects are observed.
Database (UF_D)	3	Default value of 10 was reduced to 3 due to the lack of reproductive/developmental studies and data in a second species.
LOAEL to NOAEL (UF_L)	1	Default value of 10 was reduced to 1 because $BMDL_{10}$ was used as the point of departure.

An oral reference dose to apply to workers (RfD_{occ}) of 0.008 mg/kg-day was calculated by dividing the $BMDL_{10}$ (2.3 mg/kg-day) by the total UF of 300 (i.e., 3x10x3x3x1). Based on a 70 kg worker inhaling 10 m^3 of air during an 8 hour work day, working 5 days per week, 46 weeks per year, and assuming 100% absorption of DNAN, the OEL was calculated as:

$$OEL = (0.008\ mg/kg\text{-}day)(70\ kg)(day/10\ m^3)(7\ day/5\ day)(52\ wk/46\ wk) = 0.09\ mg/m^3$$

VII. RECOMMENDED OEL

0.09 mg/m^3, skin

Note 1: The OEL is the Time-weighted average (TWA) for a conventional 8-hour work day and a 40-hour work week, to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effects.

Note 2: The use of the Skin notation is intended to alert the reader that air sampling alone is insufficient to quantify exposure accurately and that measures to prevent significant cutaneous absorption may be required.

VIII. RECOMMEDATIONS

The OEL provided was derived using limited data and conventional extrapolation procedures. More data are needed to increase the confidence of extrapolating these data to humans. Examples include reproductive/developmental studies, chronic studies and human workplace exposure/effects information. Incorporation of another species into the former two suggestions is also recommended.

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